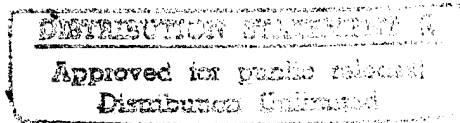


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AN INVESTIGATION OF THE POTENTIAL FOR LASER NERVE WELDING

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ABSTRACT

Suture repair of a severed peripheral nerve is cumbersome, presents a focus for infection and neuroma formation, and does not always produce adequate stump alignment. An alternative form of repair is laser nerve welding which is attractive because it does not introduce foreign material into the anastomotic site, it forms a circumferential seal, and it can be performed in difficult to reach areas. Laser repair has not been widely accepted both because the effect of laser irradiation on intact nerves is not well documented, and the anastomotic strength of the weld has been inferior to suture repair. In the first part of the present study, rat sciatic nerves were exposed and irradiated with increasing intensities from a Sharplan CO₂ and a KTP laser to document nerve damage as recorded by decreases in the peak compound action potential (CAP). A new technique of laser repair (S-Q weld) was then developed which involved harvesting subcutaneous tissue from the adjacent dermis, wrapping it around the two opposed nerve stumps, and lasering it to the epineurium to effect a weld. The strength of the S-Q weld (6.1 grams) was considerably greater than that produced by laser welding alone. The third phase of the study compared regeneration at two months in severed rat sciatic nerves repaired by either microsuture or S-Q weld. Analysis of the CAP values indicated that the number of regenerating fibers following laser repair was greater than that following suture repair, although a significant difference could not

be demonstrated. The rate of nerve dehiscence in the laser repair was unacceptably high, but the observed advantages of the technique indicate a need for further investigation.

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INTRODUCTION:

Microsuture repair is the most commonly used method for anastomosis of severed peripheral nerves. However, functional recovery following this type of repair is often inadequate even though the peripheral nervous system has a remarkable ability to regenerate adequate sizes and numbers of axons. The most significant problems with microsuture repair are inherent in the technique: 1) Surgery traumatizes the nerve by repeated introduction of a needle. 2) Suture material at the anastomotic site presents a focus for scar and neuroma formation which may impede the growth of regenerating axons from the proximal segment into the proper distal segment endoneurial tubes and, ultimately, the neuromuscular junction. 3) Microsurgical repair inevitably leaves small gaps which allow entry of fibroblasts and other scar tissue forming cells, permit regenerating axons to escape into an improper extraneural space, and promote loss of neurotrophic hormones that may be secreted locally to aid in the conduction of regenerating axons to their proper target. 4) Microsurgery is time consuming and may be difficult to perform in restricted areas (e.g. intraoral repair of lingual and mandibular nerves), particularly when supportive epineurial material is scarce (1).

Laser repair of severed peripheral nerves has been investigated by several authors, since it offers a significant theoretical improvement to the problems noted above. The mechanism of laser repair involves protein denaturation and subsequent fusion of the collagenous portion of the proximal and distal segment epineurium

by low level thermal coagulation (1). Laser application has been shown to cause a change in collagen substructure with interdigitation of altered fibrils (2). This mechanism of repair avoids the trauma imposed by needles, and it does not introduce foreign material into the anastomotic site. Furthermore, the seal formed by the laser repair is circumferential and therefore discourages entry of fibroblasts or exit of regenerating axons and neurotrophic hormones. Lastly, several authors have noted that laser welding takes significantly less time than microsuture repair, and it can be performed in difficult to reach areas (3, 4).

The advantages of laser nerve welding indicate that it has potential for clinical application. However, there are two major problems that prevent the widespread acceptance of this technique. First, the effect of laser radiation on intact nerves has not been well documented. Second, the anastomotic strength of the laser weld has been found consistently inferior to that of microsuture repair for at least the first four days following surgery (5). The weak initial weld has caused unacceptable rates of dehiscence and prompted the use of stay sutures in many of the animal models (1,3,4,6,7).

The present study attempted to improve upon the laser weld technique by first examining the effect of laser radiation on the compound action potential of intact rat sciatic nerves. In the second phase of the study, a new technique to improve anastomotic strength was developed (S-Q weld) which involved harvesting a sheet of subcutaneous tissue from the experimental animal, wrapping it around the cut nerve ends, and lasering it to the epineurium. The

final part of the study examined the long term effectiveness of the technique as compared to microsuture repair in the rat sciatic nerve model.

MATERIALS AND METHODS:

Study 1: The Effect of Laser Irradiation on Intact Nerve Function:

In this initial study, the effect of KTP, nd:YAG, and CO₂ laser irradiation on the compound action potential (CAP) of intact sciatic nerves was investigated. All animals used in these experiments were cared for in compliance with "The Principles of Laboratory Animal Care" formulated by the National Society for Medical Research. Twenty-one Sprague Dawley rats were anesthetized with an intraperitoneal injection of 0.13 cc sodium pentobarbital mixed with 0.87 cc ketamine (per Kg). The sciatic nerve of each rat was exposed and a pair of differential recording electrodes positioned just proximal to the nerve trifurcation. A pair of stimulating electrodes was then placed 3 centimeters proximal to the recording electrodes and the nerve suspended in air to improve recording isolation. Single square-wave pulses were applied to the nerve using a Grass S88 stimulator. The stimulus intensity was increased until a CAP of maximum peak to peak amplitude (P-P CAP) was recorded. The nerve was then irradiated midpoint between the electrodes using increasing laser intensities as listed in Table I. CAP recordings were obtained following the laser treatment at each intensity. Each treatment involved irradiation with 30 pulses, 0.05

seconds in duration at a spot size of 0.36 mm. A Sharplan 1060 CO₂ laser and a Laserscope KTP laser were used in these experiments. The effect of laser irradiation on nerve function at each wattage was then determined by expressing post-irradiation P-P CAP values as a percentage of pre-irradiation P-P CAP value, indicating the percent of viable fibers still remaining in the nerve.

Study 2: Tensile Strength of Repaired Nerves

a. Surgical Technique:

Twenty-five Sprague Dawley rats were anesthetized using the mixture as described previously. Employing sterile technique, the sciatic nerves were exposed bilaterally and transected 3 centimeters proximal to the trifurcation with a steel scalpel. On the left side, the nerve stumps were realigned and repaired using 2 epineural sutures of 10-0 nylon placed 180 degrees apart. On the right side, the nerve stumps were reanastomosed using the subcutaneous tissue weld technique (S-Q weld). A small piece of translucent subcutaneous tissue was harvested and stretched over a 1 cm² piece of aluminum foil. The foil was placed under the transected nerve ends. The nerve ends were reapproximated and the S-Q tissue was wrapped around the nerve and spot welded to the epineurium using a Sharplan 1060 CO₂ laser. The energy paradigm of 1.00 Watt, 0.05 sec single pulses, 0.36 mm spot size was chosen, since this appeared to represent the nominal power level for bonding subcutaneous tissue to nerve epineurium. Multiple pulses were applied to the S-Q sheath a few millimeters from the anastomotic site around a circumference of 180 degrees to allow for adequate

stump alignment with room for nerve swelling. (The S-Q sheath completely encircled the anastomotic site). All surgeries were performed by the same surgeon (M.K.) until the sheath appeared tightly bound.

In ten of the rats, the laser and suture repaired nerves were harvested immediately for tensile strength measurements. In the remaining fifteen, wounds were closed and the animals were placed in their cages with no restriction of movement for two months.

b. Measurement of Tensile Strength:

Suture, laser repaired, and intact nerves were evaluated for tensile strength. Approximately 4 cm of sciatic nerve was excised. 4-0 silk ligature was tied from one end of each nerve to a calibrated force transducer (Grass Instruments, Quincy Mass) whose output was amplified and visualized on an oscilloscope. The opposite nerve end was attached with 4-0 silk ligature to a platform mounted on a variable speed infusion pump (Harvard apparatus) which generated slow, steady tension. Tension was applied until the nerve separated, and breaking force was recorded.

Study 3: Electrophysiological and Histological Evaluation of Regeneration in Repaired Nerves.

After allowing two months for regeneration, the sciatic nerves were removed from 15 rats for CAP transmission studies and histological examination. Nerves were harvested, placed in an isolation chamber, and bathed in Ringer's solution. Bipolar stimulating electrodes were placed proximal and recording electrodes positioned distal to the anastomotic site. The P-P CAP

from the laser repaired nerve was recorded and expressed as a percentage of the P-P CAP of the suture repaired nerve in each rat. All recordings were taken with nerves suspended in air. Each nerve was then fixed in 4% paraformaldehyde buffered by .1 M phosphate (pH 7.4), post-fixed in osmium tetroxide, alcohol dehydrated, imbedded in epon, and thin sectioned for histological and E.M. analysis.

Results:

The effects of laser irradiation on compound action potential transmission are presented in Table I. The nd:YAG laser was dropped from the study because it caused significant endoneurial damage at nominal intensities, as evidenced by CAP depression. As can be seen from the table, both the CO₂ and the KTP lasers produced almost no decrease in P-P CAP transmission at 0.5 watts. However, this level of irradiation did not provide adequate bonding of subcutaneous tissue to nerve epineurium during S-Q welding in the later two studies. In these studies, a higher power of 1.0 watt was used, since it produced adequate bonding with minimal anticipated increase in damage to endoneurial axons and support structures. It was estimated that considerably less than 40% of the radiation penetrated the endoneurium in these studies, corresponding to 60% remaining axon function in Table 1, since some of the radiation was absorbed by the subcutaneous tissue wrap. Intensities as high as 2.5 watts were often sufficient to cut a nerve in half as indicated in the table. In such instances, the P-P CAP value was recorded as zero.

The results of the tensile strength study are presented in Figure I. Intact nerves (n=11) showed a breaking force of 218.6 gms \pm 14.6. Suture repaired nerves (n=10) showed a breaking force of 47 gms \pm 10.8, which represented 21.5% of the intact nerve strength. The S-Q Weld technique (n=10) had a breaking force of 6.1 gms \pm 5.0, which represented 2.8% of the intact nerve strength.

At two months post surgery, 6 of 15 rats had intact laser anastomosis while 14 of 15 had intact suture anastomosis. The P-P CAP value from each laser repaired nerve was recorded and expressed as a percentage of the P-P CAP of the suture repaired nerve. The average ratio for all animals measured was 1.05 ± 0.18 , indicating that the number of regenerating fibers following laser repair was greater than that following suture repair, although a significant difference could not be demonstrated. Histological examination of nerve segments distal to the laser or suture repair sites demonstrated no significant differences in the numbers of axons or their population diameters (Figure II).

Discussion:

There is a wide range of reported energy paradigms for laser welding of severed peripheral nerves in animal models. Fischer et. al. used the CO₂ laser on the rat sciatic nerve at a power of 5 watts (0.6 mm spot size, 0.5 sec pulses (4), while Maragh et. al. used a power of 90-95 mW (0.2 mm spot size, 2 msec pulses (5). Our initial study was undertaken to determine an energy paradigm that would create a strong anastomosis without inflicting excessive damage upon the regenerative elements of a severed peripheral

nerve. This study assumes that irradiation which reduces the CAP of an intact nerve would also damage the endoneurial tubes in a traumatized or severed nerve. The endoneurial tubes serve as conduits to regenerating nerve fibers and are necessary in the reestablishment of appropriate target connections. A power setting of 1.0 watt was chosen, since this wattage could produce adequate welds with minimal endoneurial damage.

Inadequate tensile strength of laser anastomosis has been a problem in most of the experimental trials that have been reported. Benke et. al. reported that the tensile strength of rat sciatic nerves repaired using only the CO₂ laser was less than 10% as strong as normal nerves immediately following the repair. They concluded that the CO₂ laser weld alone does not provide enough tensile strength to be practical, and they employed the use of two stay sutures for laser anastomosis (7). Fischer et. al. used one stay suture for repair of rat sciatic nerves with the CO₂ laser, and then removed the suture after laser welding unless they felt removal would compromise the repair. They observed a dehiscence rate of 13% (4 of 31 rats) in laser repaired nerves 60 days postoperatively (4). Maragh et. al. used no stay sutures in laser repair of rat sciatic nerves, but observed a 12% dehiscence rate (2 of 17 rats) at 2 months postoperatively (5).

Other researchers have used the rabbit facial nerve model (1), the rabbit peroneal nerve model (6), and the Rhesus monkey peroneal nerve model (3). All used either stay sutures for support of laser welds or nerve grafts to reduce tension at the anastomotic site, or they observed nerve dehiscence following laser repair. These

adaptations to increase bonding strength compromise nerve regeneration. As stated earlier, suture material presents a focus for scar and neuroma formation, and nerve grafts add the additional complication of two anastomotic sites.

The S-Q weld technique employs host tissue, which is allographic, biocompatible, and readily available to provide an instrument for sealing and binding severed nerve stumps. Using this approach, the laser energy is focused on the subcutaneous wrap at sites removed from the actual nerve juncture where scarring and neuroma formation should be avoided. The tensile strength of the S-Q weld immediately following repair was far greater than the tensile strength without the subcutaneous sheath. Indeed, nerves repaired without a sheath separated before measurements could be taken. The only other study of acute tensile strength of laser repaired rat sciatic nerves reported a breaking strength of less than 10% of intact nerves (7). The strength of S-Q welded nerves was also inferior to the tensile strength of the acute suture repair, but the improvement over CO₂ laser weld alone was substantial and would encourage further refinement of this procedure. As reported by Maragh et. al. in the rat sciatic nerve model, there was no significant difference in tensile strength of the laser repaired nerves and the suture repaired nerves at 8 days postoperatively (5). The critical period is clearly the first week before host connective tissue elements add the necessary stability. In order to make laser repair an attractive alternative to suture repair, we feel that the tensile strength of the laser repaired nerve must be improved further without the use of stay sutures or grafts, which may impair

the healing process.

The rate of dehiscence for laser repair at two months postoperatively was disappointing. The nerve separation may have been due to an inordinate amount of movement of the rats in the cages. However, it is significant to note that when the anastomosis remained intact, the quality of nerve regeneration may have been superior to that following suture repair, as demonstrated by the CAP transmission studies.

We conclude that laser nerve repair employing the S-Q weld technique has several theoretical advantages over suture repair. The observed improvement in initial anastomotic strength over laser repair alone warrants the need for further investigation with different laser energies and other improvements in technique.

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FIGURE LEGENDS:

Figure I: Breaking force of normal, suture, and laser repaired nerves immediately following repair.

Table I: Percent function of nerves following laser irradiation is expressed as the ratio between post laser P-P CAP and pre laser P-P CAP. Increasing wattages were applied to the same nerve after a one minute interval.

Figure II: Brightfield micrographs of cross sections taken from the distal segment of an S-Q weld repaired nerve (A) and a suture repaired nerve (B). There was no significant difference in the number of axons in the two preparations.

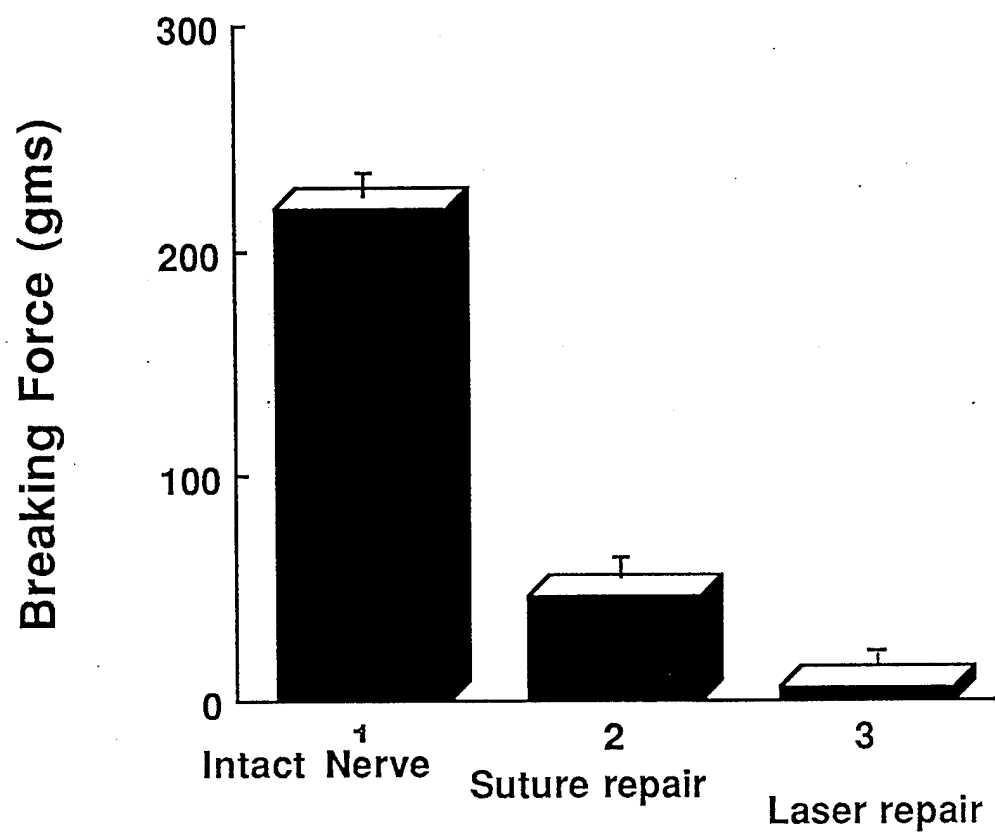


Fig. 1

TABLE 1: THE EFFECTS OF LASER IRRADIATION ON NERVE FUNCTION

<u>KTP:</u>	<u>0.5 WATTS</u>	<u>1.0 WATTS</u>	<u>1.6 WATTS</u>	<u>2.5 WATTS</u>
# Trials	8	10	10	8
% Function	0.91	0.62	0.45	0.31
St. Dev.	0.08	0.26	0.20	0.26
 <u>CO₂:</u>	 <u>0.5 WATTS</u>	 <u>1.0 WATTS</u>	 <u>1.6 WATTS</u>	 <u>2.5 WATTS</u>
# Trials	13	13	13	10
% Function	0.91	0.59	0.57	0.09
St. Dev.	0.16	0.19	0.24	0.20



Fig 2A

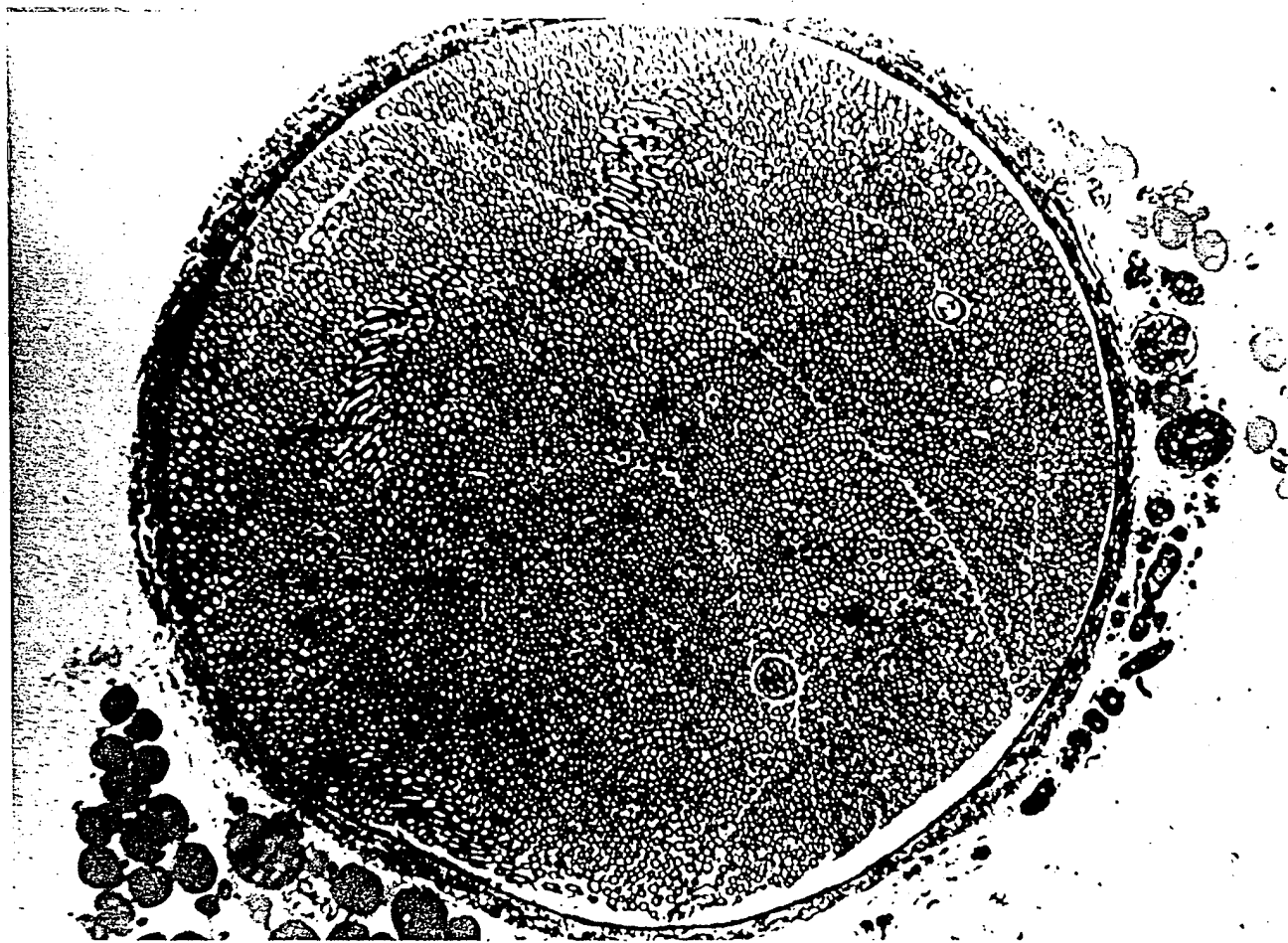


Fig 2B